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(57) Abstract

Compounds that inhibit CD8 mediated T cell activation and that have a molecular surface that corresponds to the molecular surface of human CD8 at amino acids 73–76 and/or 38–46 and/or 53–56 and/or 60–67 and pharmaceutical compositions comprising such compounds are disclosed. Method of inhibiting activation of a human T cell are disclosed. The methods comprise contacting a T cell with a compound that inhibits CD8 mediated T cell activation and that has a molecular surface that corresponds to the molecular surface of human CD8 at amino acids 73–76 and/or 38–46 and/or 53–56 and/or 60–67. Methods of treating an individual suspected of suffering from or susceptible to graft versus host disease and/or organ rejection are disclosed. The methods comprise administering an effective amount of a compound that inhibits CD8 mediated T cell activation and that has a molecular surface that corresponds to the molecular surface of human CD8 at amino acids 73–76 and/or 34–46 and/or 53–56 and/or 60–67.

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CD8 ANTAGONISTS

FIELD OF THE INVENTION

The present invention relates to molecules which interfere with CD8-mediated activity.

BACKGROUND OF THE INVENTION

Clinical allogenic bone marrow transplantation is an important therapeutic treatment for several diseases including high risk leukemia, aplastic anemia, and severe combined immunodeficiency. In addition, there is a wide range of metabolic and genetic disorders that can potentially be corrected by this approach. However, the usefulness of marrow transplantation is currently limited by several important risk factors, the principal one being graft-versus-host disease (GVHD), an often times lethal complication which occurs in a high proportion of transplants (see Korngold, R., *Amer. J. Ped. Hematol. & Oncol.* **15**:18 (1993)).

The risk of GVHD can be reduced by HLA matching of the marrow donor and recipient, with a matched sibling being the primary choice. Yet, less than 30% of the patients in North America have an HLA-matched sibling, and therefore must seek suitable unrelated HLA-matched donors from the National Marrow Donor Program. The probability of finding an unrelated HLA-matched donor is currently on the order of 30-40% and depend on the total number of donors registered. In both related and unrelated HLA-matched transplant situations, the risk of GVHD is still quite high due to disparity of

non-HLA multiple minor histocompatibility (H) antigens. GVHD is somewhat higher in unrelated cases, as this increases the probability of differences at these loci.

Mature donor T cells contaminating the marrow inoculum are responsible for GVHD. Several studies have shown that depletion of these T cells significantly diminishes the incidence of disease. However, the elimination of donor T cells has also resulted in a greater incidence of leukemic relapse. It seems important to provide at least some level of T cell immunocompetency in these completely immunocompromised patients to not only combat residual leukemia cells but also to counter opportunistic infections. In this respect, the same GVHD-reactive donor T cells may be important for targeting leukemia cells expressing the same host allogeneic histocompatibility antigens. Therapeutic approaches that could ameliorate the pathogenic tissue destruction accompanying GVHD, particularly in the gut and skin, but that would allow for continued anti-leukemia activity would greatly benefit marrow transplant patients.

Other transplantation procedures involving the implantation of immunogenic tissue include but are not limited to, heart transplants, liver transplants, kidney transplants, lung transplants, islet transplants, cornea transplants and skin grafts. In such organ and tissue transplant procedures, rejection of the transplant is an obstacle to complete recovery. The individual's immune system recognizes antigens (HLA or minor H antigens) on the implanted tissue as foreign and mounts an immune response against it which injures and destroys the implanted tissue.

T cells act as effectors of the immune response. One of the most striking ways in which they do so is by targeting cells displaying foreign antigen. The subset of T cells that mediate this lytic function are designated as cytotoxic T lymphocytes (CTL). The highly specific nature of the CTL response is apparent in cell-mediated responses to viral infections and to allografts. This sub-population of lymphocytes is characterized by expression of the cell surface marker CD8. The CD8 protein has been shown to play a major role in both activation of mature T-cells and the thymic differentiation process that leads to expression of CD8. Classically, CD8 has been viewed as an accessory molecule involved in ligation of class I major histocompatibility complex (MHC) bearing antigen on an antigen presenting cell (APC). In recent years, accumulating evidence suggests that this model for the role of CD8 in T cell activation is not complete. It is now believed that CD8

plays a major role in signal pathways leading to T cell proliferation (for review, see.Miceli and Parnes, *Adv. In Immuno.* **53**:59-72 (1993)).

CD8 has been shown to physically associate with the T cell receptor complex (TCR), as demonstrated by co-immunoprecipitation and by co-capping experiments (Gallagher et al., *PNAS* 86:10044-10048 (1989)). TCR signaling and TCR mediated lymphokine production are markedly enhanced with CD8-TCR aggregation. Characterization of the CD8 structure by a panel of monoclonal antibodies directed against CD8 showed that MHC class I binding and TCR interaction are associated with distinct regions of the CD8 molecule (Eichmann et al., *J. of Immuno.* **147**:2075-2081 (1991). In addition, CD8 and the TCR recognize the same class I molecule (Connoly et al., *PNAS* **87**:2137-2141 (1990)).

The human CD8 molecule is expressed either as an α/α homodimer or as an α/β heterodimer. Individual human peripheral T-cells can express varying amounts of CD8 α/α and α/β complexes, and their relative ratios appear to be differentially regulated upon T-cell activation. The biological activity of CD8 has primarily been attributed to the α chain, which enhances or reconstitutes T-cell responses in the homodimeric form. In contrast, until recently, no role had been ascribed to the β chain. Mice that were chimeric for the homozygous disruption of the CD8 β gene developed normally to the CD4+ CD8+ stage, but did not efficiently differentiate further, which results in a low number of peripheral CD8+ T-cells. The fact that the number of peripheral CD8+ T-cells was restored upon transfer of exogenous CD8 β gene indicates that CD8 β is necessary for the maturation of CD8+ T-cells. It has also been shown that CD8 α/β transfectants produce more IL-2 than CD8 α/α transfectants in response to specific stimuli (Wheeler et al., *Nature* **357**:247-249 (1992)). T-cell activation results in the physical modification of the mouse CD8 β chain shown by the reversible alteration in its sialic acid content (Casabo et al., *J. of Immuno.* **152**:397-404 (1994)). This modification may influence the physical structure of the CD8 complex and in turn the interaction with TCR and MHC class I. The gene encoding the CD8 molecule has been cloned for several species (human, rat, mouse) (Sukhame et al., *Cell* **40**:591-597 (1985); Nakauchi et al, *PNAS* **82**:5126-5130 (1985)). The murine CD8 molecule is expressed as a heterodimeric structure consisting of two disulfide linked subunits; Lyt-2, which has a molecular weight of about 38 kDa and Lyt-3,

which has a molecular weight of 30 kDa (Ledbetter et al., *J. of Exp. Med.* **153**:1503-1516 (1981)). The α chain gene can also undergo an alternative mode of mRNA splicing resulting in expression of the α' form which is distinguishable from α by its shorter cytoplasmic tail (Zamoyska et al., *Nature* **342**:278 (1989); Giblin et al., *PNAS* **86**:998-1002 (1989)).

Sequence analysis of CD8 indicates that it is a member of the immunoglobulin (Ig) superfamily. Members of the Ig-superfamily exhibit highly conserved hydrophobic cores. The CD8 molecule consists of an unique amino-terminal Ig-variable domain, an extracellular spacer which carries the structural features of Ig hinge-line region, a transmembrane domain and an intracellular cytoplasmic tail. The crystal structure of the extracellular Ig-like portion of the homodimeric human CD8α has been recently solved (Leahy et al., *Cell* **68**:1145-1162 (1992)). The amino-terminal domain of the CD8α chain was shown to closely resemble an Ig-variable region. The regions that are analogous to antigen-binding domains on an immunoglobulin protein are referred to as the complementarity determining regions (CDRs). Recent mutagenesis studies of the different domains of CD8 has indicated that CDR1 and CDR2 like domains are involved in MHC class I interactions (Sanders et al., *J. of Exp. Med.* **174**:371-379 (1991)).

Replacement of the human CD8α CDR2-like loop by the homologous mouse sequences results in the loss of interaction of monoclonal antibodies (MAb) that are capable of inhibiting CD2-mediated Ca^{+2} increases (Franco et al, *Cellular Immuno.* **157**:341-352 (1994)). This suggests that the CDR2-like region of CD8 α-chain may be involved in regulating T-cell activation.

These data indicate that the role of CD8 in MHC class I interaction is not incidental, but required for efficient stimulation of the T cell. The CD8 molecule plays a role very similar, yet distinct, to that of CD4 in class II MHC-restricted activation. Thus, CD8 must be involved in the regulation of a complex system of modulation of signaling involving many closely related molecules.

There is a need for pharmaceutical compositions and methods which can effectively inhibit the immune responses mediated by CD8 activity. There is a need for pharmaceutical compositions for and a method of inhibiting CD8 mediated T cell

activation. There is a need for pharmaceutical compositions and methods which can effectively inhibit GVHD in individuals undergoing allogeneic bone marrow transplantation and grafting procedures. There is a need for pharmaceutical compositions and methods which can effectively inhibit organ and tissue rejection in individuals undergoing transplantation and grafting procedures.

SUMMARY OF THE INVENTION

The present invention relates to compounds that compete with CD8 in intermolecular interactions that involve CD8 which are associated with CTL activation. The compounds comprise a molecular surface that corresponds to a molecular surface of human CD8 at amino acids 73-76, 38-46, 53-56, 60-67, or 53-67. The compounds compete with CD8 in intermolecular interactions. By competing with CD8, the compounds inhibit CD8 from participating is such intermolecular interactions and thereby inhibits CTL activation. By competing with CD8 but not activating CTL, the compounds of the invention are CD8 antagonists.

The present invention relates to peptides consisting of:

a) less than 25 amino acids, and

b) an amino acid sequence comprising at least CD8 amino acids 73-76 or CD8 amino acids 38-46 or CD8 amino acids 53-56 or CD8 amino acids 60-67; wherein the peptide inhibits CD8 activity.

The present invention relates to pharmaceutical composition comprising:

a) a peptide consisting of

i) less than 25 amino acids, and

ii) an amino acid sequence comprising at least CD8 amino acids 73-76 or CD8 amino acids 38-46 or CD8 amino acids 53-56 or CD8 amino acids 60-67, wherein said peptide inhibits CD8 activity; and

b) a pharmaceutically acceptable carrier or diluent.

The present invention relates to methods of inhibiting human CTL activation comprising contacting human CTL with a compound that competes with CD8 in intermolecular interactions that involve CD8 which are associated with CTL activation. The compound comprises a molecular surface that corresponds to a molecular surface of

human CD8 at amino acids 73-76, 38-46, 53-56, 60-67, or 52-67. The compound competes with CD8 in intermolecular interactions, thus inhibiting CD8 from participating in such intermolecular interactions and thereby inhibiting CTL activation.

The present invention relates to methods of inhibiting human CTL activation comprising contacting human CTL with a peptide consisting of:

a) less than 25 amino acids, and

b) an amino acid sequence comprising at least CD8 amino acids 73-76 or CD8 amino acids 38-46 or CD8 amino acids 53-56 or CD8 amino acids 60-67, wherein said peptide inhibits CD8 activity.

The present invention relates to methods of treating individuals who are about to undergo, are undergoing and/or have undergone transplantation procedures comprising the step of:

administering to an individual an effective amount of a compound that competes with CD8 in intermolecular interactions that involve CD8 which are associated with CTL activation. The compound comprises a molecular surface that corresponds to a molecular surface of human CD8 at amino acids 73-76, 38-46, 53-56, 60-67, or 53-67. The compound competes with CD8 in intermolecular interaction, thus inhibiting CD8 from participating is such intermolecular interactions and thereby inhibiting CTL activation.

The present invention relates to methods of treating individuals who are about to undergo, are undergoing and/or have undergone transplantation procedures comprising the step of:

administering to an individual an effective amount of a peptide consisting of:

a) less than 25 amino acids, and

b) an amino acid sequence comprising at least CD8 amino acids 73-76 or CD8 amino acids 38-46 or CD8 amino acids 53-56 or CD8 amino acids 60-67; wherein the peptide inhibits CD8 activity.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows data from experiments described in Example 9 testing the effect of the CD8 inhibitor 1109 in the CD8-dependent MHC class I - restricted skin transplantation model.

Figure 2 shows data from experiments described in Example 9 testing the ability of the CD8 inhibitor 1109 to inhibit GVHD in minor histocompatibility mismatch which is primarily CD8+ T cell mediated.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the term "CD8" is meant to refer to human CD8 protein unless otherwise specifically designated as CD8 derived from a different species. The sequence of human CD8 protein is known and is disclosed in Littman et al., *Cell* **40**:237-246 (1985). The sequence of the extracellular domain of the α chain of human CD8 is shown in SEQ ID NO:1. When CD8 amino acids are referred to by CD8 amino acid number, the number is determined from SEQ ID NO:1. The sequence of murine CD8 protein is known and is disclosed in Zamoyska et al., *Cell* **43**:153-163 (1985) and Nakauchi et al., *PNAS* **84**:4210-4214.

The present invention provides compounds that compete with CD8. Specifically, regions of human CD8 α chain have been identified which interact with other molecules in the mediation of immune responses and the activation of T cells. The compounds of the present invention effectively inhibit the immune responses mediated by CD8 activity. The compounds of the present invention effectively inhibit GVHD in individuals undergoing allogeneic bone marrow transplantation procedures. The compounds of the present invention inhibit CD8 mediated CTL activation. The compounds of the present invention effectively inhibit immune responses associated with organ and/or tissue rejection in individuals undergoing organ and/or tissue transplantation procedures.

The compounds of the present invention are CD8 antagonists. The compounds comprise a molecular surface that corresponds to the molecular surface of human CD8 α chain at amino acids 73-76, 38-46, 53-56, 60-67, or 53-67. However, while the compounds competitively interact with the molecules that CD8 interacts with at the same site that CD8 does by mimicking the surface of human CD8 at amino acids 73-76,

38-46, 53-56, 60-67, or 53-67, the interaction by the compounds does not result in CTL activation. Furthermore, the compounds, by competing with CD8, inhibit CTL activation. Thus, the CD8 antagonist compounds compete with native CD8 but do not produce the same biological effect. Accordingly, the CD8 antagonist compounds of the invention are effective inhibitors of CD8-mediated biological activity.

As used herein, the term "corresponds" is meant to refer to a similarity in structure which is sufficient to result in mimicry of activity. That is, the molecular surface of the compounds of the invention have a surface whose structure interacts with the surface molecules that CD8 interacts with in the same manner. The structure of a molecular surface is the result of amino acid side chains and the forces place upon them by the adjacent groups and the conformation of the backbone. In some embodiments, corresponding surfaces are essentially identical. In some embodiments, peptides have amino acid sequences identical to CD8 amino acid sequences. In some embodiments, peptides have amino terminal and carboxy terminal cysteines and all remaining amino acid sequences identical to CD8 amino acid sequences.

As used herein, the term "substantially similar" is meant to refer to amino acid sequences which are either identical, or not identical but which have one or more amino acid deletion, insertions and/or substitutions but remain sufficiently structurally alike such that they substantially similar sequence has a molecular surface which confers the binding properties of the native sequence.

The present invention provides compounds that display the same molecular surface as that which is displayed by amino acids 73-76 and/or 38-46 and/or 53-56 and/or 60-67 of human CD8. By displaying the same molecular surface as regions of human CD8, the compounds of the present invention interact with the same molecules as CD8 amino acids 73-76 and/or 38-46 and/or 53-56 and/or 60-67. The compounds of the present invention do not produce the same biological effect that is produced by CD8 intermolecular interaction.

According to some embodiments of the present invention, portions of the CD8 molecule which include residues 73-76 (SEQ ID NO:15) 38-46 (SEQ ID NO:11), 37-47 (SEQ ID NO:13), 52-57 (SEQ ID NO:9), 53-56 (SEQ ID NO:2), and/or 60-67 (SEQ ID NO:3), alone or linked to additional CD8 and/or non-CD8 sequences, are included in small

peptides and form a surface that participates in intermolecular interactions in competition with native CD8 amino acids 73-76 and/or 38-46 and/or 53-56 and/or 60-67. The present invention also provides small peptides which have a molecular surface that is substantially similar to the molecular surface formed by CD8 amino acids 73-76 and/or 38-46 and/or 53-56 and/or 60-67 in human CD8 but which include one or more conservative substitutions of CD8 residues 73-76 and/or 38-46 and/or 53-56 and/or 60-67. When interacting with molecules which interact with CD8, the compounds of the invention do not produce the same biological effect on cells as that which occurs through CD8 interactions. The compounds of the invention compete with and displace CD8, thereby reducing CD8 mediated immune responses and CTL activation.

The molecular surfaces defined by CD8 amino acids 73-76 or 38-46 or 53-56 or 60-67 are simulated by the compounds of the invention and it is the structural similarity to these surfaces that make the compounds useful in the methods of the present invention. In order for compounds to display substantially the same molecular surface as that which is displayed by CD8 at CD8 amino acids 73-76 or 38-46 or 53-56 or 60-67, compounds include those amino acid sequences in similar conformations as the amino acid sequences occur in the CD8 molecule. In some embodiments of the invention, compounds comprise CD8 amino acids 73-76 in similar conformation as they occur in CD8. In some embodiments of the invention, compounds comprise CD8 amino acids 38-46 in similar conformation as they occur in CD8. In some embodiments of the invention, compounds comprise CD8 amino acids 37-47 in similar conformation as they occur in CD8. In some embodiments of the invention, compounds comprise CD8 amino acids 53-56 in similar conformation as they occur in CD8. In some embodiments of the invention, compounds comprise CD8 amino acids 52-57 in similar conformation as they occur in CD8. In some embodiments of the invention, compounds comprise CD8 amino acids 60-67 in similar conformation as they occur in CD8. Compounds may comprise additional amino acids or molecular entities or moieties provided the active sequence, i.e. CD8 amino acids 73-76 or 38-46 or 53-56 or 60-67, is in an active conformation, i.e. a similar conformation as the sequence that is present in CD8. In some contemplated embodiments, some or all of the amino acids in the active sequence are substituted with conservative substitutions of amino acids.

In some embodiments of the invention, compounds comprise CD8 amino acids 73-76 in similar conformation to that in which they occur in CD8. In these embodiments, compounds may comprise additional amino acids or molecular entities or moieties provided the active sequence, CD8 amino acids 73-76, is in an active conformation, i.e. a similar conformation as the sequence that is present in CD8. In some contemplated embodiments, some or all of the amino acids in the active sequence are substituted with conservative substitutions of amino acids. For example, amino acid 73 is preferably leucine but can be substituted with isoleucine or valine. Amino acid 74 is preferably glycine but, as noted, conservative substitutions therefor are within the scope of the invention. For example, amino acid 74 may be phenylalanine. Amino acid 75 is preferably aspartic acid but can be substituted with, for example, glutamic acid or asparagine. Amino acid 76 is preferably threonine but can be substituted with, for example, serine. Amino acid sequences including these exemplary substitutions include: SEQUENCE ID NO:15 through SEQUENCE ID NO:28.

According to some preferred embodiments of the present invention, the peptides contain CD8 amino acids 73-76, SEQ. ID NO: 15, and terminal linking moieties. The peptide is useful in the treatment of GVHD. Exemplary suitable linking moieties include cysteine, phenylalanine, penicillamine, proline and glycine. Preferred linking moieties are cysteine but linking groups can include any organic moiety which can cyclicize the amino acid sequence. In a highly preferred embodiment the peptide is a conformationally restricted cyclic peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 73-76 but it, SEQ ID NO:15, will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 53-56, SEQ ID NO:2, are used in peptides of the invention. Peptides of some embodiments of the present invention consist of amino acids 53-56 plus amino terminal and carboxy terminal cysteines, SEQ ID NO:4. Peptides of other embodiments of the present invention consist of amino acids 73-76 plus amino terminal and carboxy terminal cysteines. The peptide is useful in the treatment of GVHD. The peptide is a conformationally restricted cyclic

peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 53-56 but it, SEQ ID NO:4, will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 53-56, SEQ ID NO:2, are used in peptides of the invention. Peptides in some embodiments of the invention consist of amino acids 53-56 plus an amino terminal cysteine and a carboxy terminal proline-cysteine to form SEQ ID NO:5. The peptide is useful in the treatment of GVHD. The peptide is a conformationally restricted cyclic peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 53-56 but it, SEQ ID NO:5, will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 60-67, SEQ ID NO:3, are used in peptides of the invention. Peptides that consist of amino acids 60-67 plus amino terminal and carboxy terminal cysteines, SEQ ID NO:6, are an effective inhibitor of CD8 mediated T cell activation. As such, the peptide is useful in the treatment of GVHD. The peptide is a conformationally restricted cyclic peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 60-67 but it, SEQ ID NO:6, will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 52-57, SEQ ID NO:9, are used in peptides of the invention. Peptides in some embodiments of the invention consist of amino acids 52-57 plus an amino terminal cysteine and a carboxy terminal cysteine to form SEQ ID NO:10. The peptide is useful in the treatment of GVHD. The peptide is a conformationally restricted cyclic peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 52-57, but it will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 71-78, SEQ ID NO:45, are used in peptides of the invention. Peptides that consist of amino acids 71-78 plus amino terminal and carboxy terminal cysteines are effective inhibitors of CD8 mediated T cell activation. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 71-78, but it will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 38-46, SEQ ID NO:11, are used in peptides of the invention. Peptides of some embodiments of the present invention consist of amino acids 38-46 plus amino terminal and carboxy terminal cysteines, SEQ ID NO:12. The peptide is useful in the treatment of GVHD and in inhibiting rejection in skin transplantation procedures. The peptide SEQ ID NO:11 is linear while SEQ ID NO:12 is a conformationally restricted cyclic peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The peptides display substantially the same surface as CD8 at amino acids 38-46 but will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 37-47, SEQ ID NO:13, are used in peptides of the invention. Peptides of some embodiments of the present invention consist of amino acids 37-47 plus amino terminal and carboxy terminal cysteines, SEQ ID NO:14. The peptide is useful in the treatment of GVHD and in inhibiting rejection in skin transplantation procedures. The peptide SEQ ID NO:13 is linear while SEQ ID NO:14 is a conformationally restricted cyclic peptide cyclicized by a disulfide bond formed between the amino terminal and carboxy terminal cysteines. The peptides display substantially the same surface as CD8 at amino acids 33-47 but will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

According to some preferred embodiments, CD8 amino acids 71-78, SEQ ID NO:45, are used in peptides of the invention. Peptides that consist of amino acids 71-78 plus amino terminal and carboxy terminal cysteines, SEQ ID NO:31, are effective inhibitors of CD8 mediated T cell activation. Such a peptide is useful in the treatment of GVHD. The peptide is a conformationally restricted cyclic peptide cyclicized by a

disulfide bond formed between the linking groups. Preferred linking groups include amino terminal and carboxy terminal cysteines. The conformationally restricted peptide will display substantially the same surface as CD8 at amino acids 71-78 will not produce the same biological effect as that which is identified with CD8 intermolecular interactions.

The present invention relates to molecules that display the same surface as CD8 at amino acids 73-76 and/or 38-46 and/or 53-56 and/or 60-67 and which inhibit CD8 mediated immune responses such as CD8 mediated T cell activation.

The present invention relates to molecules that display the same surface as CD8 at amino acids 73-76 and/or 38-46 and/or 53-56 and/or 60-67 and which do not produce the same biological effect as CD8 does when participating in intermolecular interactions. The discovery that molecules that display the same molecular surface as is displayed by CD8 amino acids 73-76 or 38-46 or 53-56 or 60-67 provides the information necessary to create molecules which are effective inhibitors of CD8 mediated immune responses such as CD8 mediated T cell activation. Specifically, molecules of the present invention display the molecular surface that is displayed by CD8 at amino acids 73-76 or 38-46 or 53-56 or 60-67. The remaining portions of the molecules of the present invention are confined to those which do neither impede the molecule from assuming the correct conformation to form the proper molecular surface nor present interfering surfaces which would prevent the molecule from interacting similar to CD8.

According to some embodiments of the present invention, the invention can be represented by the formula:

$$R_1 - R_2 - R_3 - R_4 - R_5$$

wherein:

R_1, R_2, R_3, R_4, and R_5 taken together are 25 amino acids or less and

R_1 is a linking moiety, preferably cysteine or penicillamine;

R_2 is 0-10 amino acids, preferably 0 amino acids;

R_3 is SEQ ID NO:2, SEQ ID NO:3, an amino acid sequence comprising both SEQ ID NO:2 and SEQ ID NO:3, SEQ ID NO:9, an amino acid sequence comprising both SEQ ID NO:9 and SEQ ID NO:3, or SEQ ID NO:11 or SEQ ID NO:13;

R_4 is 0-10 amino acids, preferably 0 amino acids; and

R_5 is a linking moiety, preferably cysteine or penicillamine.

In some preferred embodiments, R_1 is cysteine or penicillamine. It is more preferred that R_1 is cysteine.

In some preferred embodiments, R_2 is 0 amino acids.

In some preferred embodiments, R_3 is SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:9 or SEQ ID NO:11 or SEQ ID NO:13.

In some preferred embodiments, R_4 is 0-1 amino acids. In some embodiments, R_4 is proline, It is preferred that R_4 is 0 amino acids.

In some preferred embodiments, R_5 is cysteine or penicillamine. It is more preferred that R_5 is cysteine.

In some preferred embodiments, the compound is SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:10, SEQ ID NO:11 or SEQ ID NO:12 or SEQ ID NO:13.

In some embodiments, SEQ ID NO:2 or SEQ ID NO:3 or SEQ ID NO:9 or SEQ ID NO:11 may contain conservative substitutions.

In some preferred embodiments, peptides have the following formula.

$$R_{11} - R_{12} - R_{13} - R_{14} - R_{15} - R_{16}$$

wherein:

R_{11} is cysteine or penicillamine;

R_{12} is serine or threonine;

R_{13} is glutamine or asparagine;

R_{14} is asparagine or glutamine;

R_{15} is lysine, hydroxylysine, proline-lysine or proline-hydroxylysine; and,

R_{16} is cysteine or penicillamine.

In some preferred embodiments, R_{11} is cysteine. In some preferred embodiments, R_{12} is serine. In some preferred embodiments, R_{13} is glutamine. In some preferred embodiments, R_{14} is asparagine. In some preferred embodiments, R_{15} is lysine or proline-lysine. In some preferred embodiments, R_{16} is cysteine. In some preferred embodiments, R_{11} is cysteine, R_{12} is serine, R_{13} is glutamine, R_{14} is asparagine, R_{15} is lysine and R_{16} is cysteine (SEQ ID NO:4). In some preferred embodiments, R_{11} is cysteine, R_{12} is serine, R_{13} is glutamine, R_{14} is asparagine, R_{15} is proline-lysine and R_{16} is cysteine (SEQ ID NO:5).

In some preferred embodiments, peptides have the following formula.

R_{21} - R_{22} - R_{23} - R_{24} - R_{25} - R_{26} - R_{27} - R_{28} - R_{29} - R_{30}

wherein:

R_{21} is cysteine or penicillamine;

R_{22} is alanine, valine, isoleucine, leucine or glycine;

R_{23} is glutamic acid or aspartic acid;

R_{24} is glycine, valine, isoleucine, leucine or alanine;

R_{25} is leucine, valine, isoleucine, alanine or glycine;

R_{26} is aspartic acid and glutamic acid;

R_{27} is threonine and serine;

R_{28} is glutamine or asparagine;

R_{29} is arginine; and,

R_{30} is cysteine or penicillamine.

In some preferred embodiments, R_{21} is cysteine. In some preferred embodiments, R_{22} is alanine. In some preferred embodiments, R_{23} is glutamic acid. In some preferred embodiments, R_{24} is glycine. In some preferred embodiments, R_{25} is leucine. In some preferred embodiments, R_{26} is aspartic acid. In some preferred embodiments, R_{27} is threonine. In some preferred embodiments, R_{28} is glutamine. In some preferred embodiments, R_{30} is cysteine. In some preferred embodiments, R_{21} is cysteine, R_{22} is alanine, R_{23} is glutamic acid, R_{24} is glycine, R_{25} is leucine, R_{26} is aspartic acid, R_{27} is threonine, R_{28} is glutamine, R_{29} is arginine and R_{30} is cysteine (SEQ ID NO:6).

In some embodiments, such as those set forth below, peptides include CD8 amino acids 73-76 (SEQ ID NO:15), or those having substitutions such as those set forth in SEQ ID NOS: 16-28. Each of the formulae set forth below includes CD8 amino acids 73-76 as R_{104}-R_{107}, and may include SEQ ID NO: 15 or one of SEQ ID NOS: 16-28.

In some preferred embodiments, peptides of the present invention have the formula:

R_{101}-R_{102}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{102} is optional and, if present, lysine, arginine, valine or histidine;

R_{103} is optional and, if present, arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is optional and, if present, phenylalanine, tyrosine, tryptophan or valine;

R_{109} is optional and, if present, valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. Preferably, each of R_{102}, R_{103}, R_{108} and R_{109} are present. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, R_{108} is phenylalanine, and R_{109} is valine. According to this embodiment, the compound has SEQ ID NO:31 and is cyclicized by the terminal cysteines. In other preferred embodiments, peptides have amino acid sequences identical to SEQ ID NO:31 except having one or more conservative amino acid substitutions. The peptide set forth in SEQ ID NO:32 is identical to that of SEQ ID NO:31 except in SEQ ID NO:32, R_{102} is histidine. The peptide set forth in SEQ ID NO:33 is identical to that of SEQ ID NO:31 except in SEQ ID NO:33, R_{102} is arginine. The peptide set forth in SEQ ID NO:34 is identical to that of SEQ ID NO:31 except in SEQ ID NO:34, R_{102} is arginine and R_{103} is lysine. The peptide set forth in SEQ ID NO:35 is identical to that of SEQ ID NO:31 except in SEQ ID NO:35, R_{102} is arginine and R_{103} is glutamine. The peptide set forth in SEQ ID NO:36 is identical to that of SEQ ID NO:31 except in SEQ ID NO:36, R_{102} is histidine and R_{103} is glutamine. The peptide set forth in SEQ ID NO:37 is identical to that of SEQ ID NO:31 except in SEQ ID NO:37, R_{102} is histidine and R_{103} is lysine. The peptide set forth in SEQ ID NO:38 is identical to that of SEQ ID NO:31 except in SEQ ID NO:38, R_{109} is leucine. The peptide set forth in SEQ ID NO:39 is identical to that of SEQ ID NO:31 except in SEQ ID NO:39, R_{109} is isoleucine. The peptide set forth in SEQ ID NO:40 is identical to that of SEQ ID NO:31 except in SEQ ID NO:40, R_{108} is tyrosine. The peptide set forth in SEQ ID NO:41 is identical to that of SEQ ID NO:31 except in SEQ ID NO:41, R_{108} is tryptophane. The peptide set forth in SEQ ID NO:42 is identical to that of SEQ ID NO:31 except in SEQ ID NO:42, R_{108} is

tryptophane and R_{109} is isoleucine. The peptide set forth in SEQ ID NO:43 is identical to that of SEQ ID NO:31 except in SEQ ID NO:43, R_{108} is tyrosine and R_{109} is leucine. The peptide set forth in SEQ ID NO:44 is identical to that of SEQ ID NO:31 except in SEQ ID NO:44, R_{108} is tyrosine and R_{109} is isoleucine. In some embodiments, peptides are made identical to SEQ ID NOs:31-44 except instead of the native CD8 sequence R_{104}-R_{105}-R_{106}-R_{107} set forth in SEQ ID NO:15 and found in each of SEQ ID NOs:31-44, the peptides contain one of modified CD8 sequences R_{104}-R_{105}-R_{106}-R_{107} set forth in SEQ ID NOs:16-28. Accordingly, this disclosure is intended to describe each permutation of SEQ ID NOs:31-44 substituted with any one of SEQ ID NOs:16-28 at R_{104}-R_{105}-R_{106}-R_{107}.

In another preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{103} is arginine, R_{104} is alanine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, R_{108} is phenylalanine, and R_{109} is valine. According to this embodiment, the compound has SEQ ID NO:46 and is cyclicized by the terminal cysteines. In another preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is alanine, R_{107} is threonine, R_{108} is phenylalanine, and R_{109} is valine (SEQ ID NO:47). According to this embodiment, the compound is cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

$$R_{101}\text{-}R_{102}\text{-}R_{103}\text{-}R_{104}\text{-}R_{105}\text{-}R_{106}\text{-}R_{107}\text{-}R_{108}\text{-}R_{110}$$

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine,

R_{102} is lysine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{108} is phenylalanine. According to this embodiment, the compound has SEQ ID NO:48 and is cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

R_{101}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{108} is phenylalanine (SEQ ID NO:29). In this embodiment, the compound is preferably cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

R_{101}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, and R_{107} is threonine. In this embodiment, the compound has SEQ ID NO:59 and is preferably cyclicized by the terminal cysteines.

In some embodiments of the present invention, the peptides have the formula:

R_{101}-R_{104}-R_{105}-R_{106}-R_{107}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In some preferred embodiments, R_{101} and R_{110} are cysteine, R_{104} is leucine, R_{105} is glycine, R_{106} is aspartic acid, and R_{107} is threonine (SEQ ID NO:30). In this embodiment, the compound is preferably cyclicized by the terminal cysteines.

In other embodiments of the present invention, the peptides have the formula:

R_{101}-R_{102}-R_{104}-R_{105}-R_{106}-R_{107}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, and R_{107} is threonine (SEQ ID NO:50).

In some preferred embodiments, peptides of the present invention have the formula:

$$R_{101}\text{-}R_{102}\text{-}R_{104}\text{-}R_{105}\text{-}R_{106}\text{-}R_{107}\text{-}R_{108}\text{-}R_{110}$$

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{108} is phenylalanine. In this embodiment, the compound has SEQ ID NO:51 and is preferably cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

$$R_{101}\text{-}R_{102}\text{-}R_{104}\text{-}R_{105}\text{-}R_{106}\text{-}R_{107}\text{-}R_{108}\text{-}R_{109}\text{-}R_{110}$$

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, R_{108} is phenylalanine, and R_{109} is valine. According to this embodiment, the compound has SEQ ID NO:63 and is preferably cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

$$R_{101}\text{-}R_{102}\text{-}R_{104}\text{-}R_{105}\text{-}R_{106}\text{-}R_{107}\text{-}R_{109}\text{-}R_{110}$$

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{109} is valine. According to this embodiment, the compound has SEQ ID NO:57 and is preferably cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

$$R_{101}\text{-}R_{102}\text{-}R_{103}\text{-}R_{104}\text{-}R_{105}\text{-}R_{106}\text{-}R_{107}\text{-}R_{109}\text{-}R_{110}$$

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{109} is valine. According to this embodiment, the compound has SEQ ID NO:53 and is preferably cyclicized by the terminal cysteines.

In some preferred embodiments, peptides of the present invention have the formula:

R_{101}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{109} is valine. According to this embodiment, the compound has SEQ ID NO:54 and is preferably cyclicized by the terminal cysteines.

In other embodiments of the present invention, the peptides have the formula:

R_{101}-R_{102}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{102} is lysine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, and R_{107} is threonine. In this embodiment, the compound has SEQ ID NO: 49, and the compound is preferably cyclicized by the terminal cysteines.

In other embodiments of the present invention, the peptides have the formula:

R_{101}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, R_{108} is phenylalanine, and R_{109} is valine. In this embodiment, the compound has SEQ ID NO:58 and is preferably cyclicized by the terminal cysteines.

In other embodiments, the peptides h ave the formula:

R_{101}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{108} is phenylalanine. In this embodiment, the compound has SEQ ID NO:55 and is preferably cyclicized by the terminal cysteines.

In other embodiments of the invention, the peptides have the formula:

R_{101}-R_{104}-R_{105}-R_{106}-R_{107}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, and R_{109} is valine. In this embodiment, the compound has SEQ ID NO:56 and is preferably cyclicized by the terminal cysteines.

In other embodiments of the present invention, the peptides have the formula:

R_{101}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

Linking moieties R_{101} and R_{110} are preferably cysteine, phenylalanine, penicillamine, or proline, more preferably cysteine. In a preferred embodiment, R_{101} and R_{110} are cysteine, R_{104} is leucine, R_{105} is glycine, R_{106} is asparagine, R_{107} is threonine, R_{108} is phenylalanine, and R_{109} is valine. In this embodiment, the compound has SEQ ID NO:60 and is preferably cyclicized by the terminal cysteines.

Particularly preferred are embodiments, having the formulas listed above, wherein R_{101} is cysteine, R_{102} is lysine, R_{103} is arginine, R_{104} is leucine, R_{105} is glycine, R_{106} is aspartic acid, R_{107} is threonine, R_{108} is phenylalanine, R_{109} is valine, and R_{110} is cysteine (SEQ. ID NO:31).

Peptides are provided which have 4-25 amino acids, preferably 6-20 amino acids, more preferably 6-15 amino acids, even more preferably 6-12 amino acids, still more preferably 8-12 amino acids, and still even more preferably 8-10 amino acids. The peptides comprise CD8 amino acids 73-76 or 38-46 or 53-56 and/or 60-67. These peptides have a restricted conformation and the ability to inhibit CD8 mediated immune responses such as CD mediated T cell activation. The inhibition of CD8 mediated immune responses can be used as the mechanism to prevent or reduce the severity of GVHD in individuals who are undergoing or who have undergone allogenic bone marrow transplantation procedures, or to prevent or reduce rejection in individuals who are undergoing or who have undergone organ and/or tissue transplantation.

In order to maximize the overlap between the conformational repertoire of CD8 with that of the peptides of the invention, peptides have been circularized via an

artificially introduced disulfide bridge. Amino terminal and carboxy terminal cysteines have been provided which can be used for formation of disulfide bonds which cyclicize the peptide. Similarly, penicillamine may be used at the amino terminal, carboxy terminal or both to provide the necessary group to form disulfide bonds with opposing penicillamine or cysteines. With the restraints which occur when the peptides are cyclicized, the peptides adopt a folding pattern similar to that of the corresponding domain in CD8.

The present invention provides synthetic peptides that are less than 25 amino acids and comprise amino acids 73-76 or 38-46 or 53-56 and/or 60-67 of CD8. The present invention provides synthetic peptides which contain an amino acid sequence from CD8 that includes CD8 amino acids 73-76, CD8 amino acids 38-46, or CD8 amino acids 53-56 or 60-67 or both, and optionally also includes other CD8 amino acids. Non-CD8 amino acid sequences are provided in some embodiments. The peptides are conformationally restricted, and are generally cyclicized. In some embodiments, non-CD8 sequences are included to for the purposes of conformational restriction. In embodiments that comprise both CD8 and non-CD8 sequences, at least 20-25% of the amino acid sequence of the peptides of the present invention are derived from CD8 including CD8 amino acid sequence 73-76, 38-46 or 53-56 or 60-67. It is preferred that greater than about 20-25% of the amino acid sequence of the peptides of the present invention are derived from CD8, more preferably 30-40% and more preferably greater than 50%. In some embodiments, the percentage of amino acid sequence of the peptides of the present invention derived from CD8 approaches about 60% or about 75% or more.

The peptides of the present invention may be prepared by any of the following known techniques. Conveniently, the peptides may be prepared using the solid-phase synthetic technique initially described in Merrifield (1963) *J. Am. Chem. Soc.* 15:2149-2154. Other peptide synthesis techniques may be found, for example, in M. Bodanszky *et al.*, *Peptide Synthesis*, John Wiley & Sons, 2d Ed. (1976); Kent and Clark-Lewis in *Synthetic Peptides in Biology and Medicine*, p. 295-358, eds. Alitalo, K., Partanen, P. and Vakeri, A., Elsevier Science Publishers, (Amsterdam, 1985); as well as other reference works known to those skilled in the art. A summary of peptide synthesis techniques may be found in J. Stuart and J.D. Young, *Solid Phase Peptide Synthelia*, Pierce Chemical Company, Rockford, IL (1984). The synthesis of peptides by solution

methods may also be used, as described in *The Proteins*, Vol. II, 3d Ed., p. 105-237, Neurath, H. et al., Eds., Academic Press, New York, NY (1976). Appropriate protective groups for use in such syntheses will be found in the above texts, as well as in J.F.W. McOmie, *Protective Groups in Organic Chemistry*, Plenum Press, New York, NY (1973).

In general, these synthetic methods involve the sequential addition of one or more amino acid residues or suitable protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively-removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group, such as lysine.

Using a solid phase synthesis as an example, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complementary (amino or carboxyl) group suitably protected is admixed and reacted with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to provide the final peptide. The peptide of the invention are preferably devoid of benzylated or methylbenzylated amino acids. Such protecting group moieties may be used in the course of synthesis, but they are removed before the peptides are used. Additional reactions may be necessary, as described elsewhere, to form intramolecular linkages to restrain conformation.

The present peptides may also be prepared by recombinant DNA techniques, although such methods are not preferred because of the need for purification and subsequent chemical modifications to conformationally restrain the peptides.

In addition to peptides which comprise L amino acids, pharmaceutical compositions according to the present invention may comprise peptides made up of D amino acids. Because most enzymes involved in degradation recognize a tetrahedral alpha-carbon, the D-amino acids were utilized in order to avoid enzyme recognition and

subsequent cleavage. Our computer studies indicate that the same folded presentation of the peptide is accomplished by reversing the amino acid sequence, employing D-amino acids. Thus, peptides comprised of D amino acids are less susceptible to degradation.

Conservative substitutions in the amino acid sequence may be made. Those having ordinary skill in the art can readily design compounds of the invention with conservative substitutions for CD8 amino acids. For example, following what are referred to as Dayhof's rules for amino acid substitution (Dayhof, M.D. (1978) *Nat. Biomed. Res. Found.*, Washington, D.C. Vol. **5**, supp. 3), amino acid residues in a peptide sequence may be substituted with comparable amino acid residues. Such substitutions are well known and are based the upon charge and structural characteristics of each amino acid. Contemplated equivalents of the molecules of the present invention include those having conservative substitution. It is contemplated that cysteine may be substituted with penicillamine in compounds that are equivalent to those of the present invention. It is contemplated that serine may be substituted with threonine in compounds that are equivalent to those of the present invention. It is contemplated that glutamine may be substituted with asparagine in compounds that are equivalent to those of the present invention. It is contemplated that asparagine may be substituted with glutamine in compounds that are equivalent to those of the present invention. It is contemplated that lysine may be substituted with hydroxylysine, proline-lysine or proline-hydroxylysine in compounds that are equivalent to those of the present invention.

L or D amino acids may be used in the synthesis. Peptides may be synthesized with amino acid sequences in the order they occur in CD8 or in the reverse order. In peptides comprising all L amino acids, it is preferred that they are synthesized such that the amino acid sequences are assembled in the order that they occur in CD8. In peptides comprising all D amino acids, it is preferred that they are synthesized such that the amino acid sequences are assembled in the reverse order that they occur in CD8.

Synthesized peptides may be circularized in order to mimic the geometry of those portions as they occur in CD8. Circularization may be facilitated by disulfide bridges between cysteine residues. Alternatively, the peptides may be circularized by means of covalent bonds, such as amide bonds, between amino acid residues of the peptide such as those at or near the amino and carboxy termini.

Peptides for use in pharmaceutical compositions of the present invention may be designed following the guidelines set out herein and using well known processes. Methods of synthesizing peptides and circularizing them may be performed routinely using standard techniques and readily available starting materials.

The present invention relates to a method of therapeutically or prophylactically treating an individual suffering from or susceptible to graft versus host disease. Such individuals include those who are undergoing and/or who have undergone transplantation procedures such as allogeneic bone marrow transplants. Those having ordinary skill in the art can readily identify individuals suspected of suffering from or being susceptible to graft versus host disease. Those with ordinary skill in the art could readily identify individuals for whom administration of the compounds of the invention would be beneficial to alleviate or prevent immune response associated with GVHD. Treatment may be provided prophylactically in conjunction with transplantation procedure or in response to symptoms associated with GVHD. Pharmaceutical compositions useful in the methods of the present invention comprise the compounds described herein.

The method of therapeutically or prophylactically treating an individual suffering from or susceptible to GVHD comprises administering to such an individual an effective amount of a peptide according to the invention. A prophylactically effective amount is an amount which is effective to prevent or decrease the immune response associated with GVHD in an individual susceptible to GVHD. A therapeutically effective amount is an amount which is effective to decrease or eliminate GVHD in an individual suffering from GVHD. Those having ordinary skill in the art can readily and routinely determine the ranges of both prophylactically and therapeutically effective amounts of the peptides of the invention without undue experimentation.

The present invention relates to methods of therapeutically or prophylactically treating an individual suffering from or susceptible to organ and/or tissue rejection. Such individuals include those who are undergoing and/or who have undergone organ and/or tissue transplant procedures such as, for example, liver transplants, heart transplants, kidney transplants, lung transplants, islets transplants, cornea transplants, bone marrow transplants and skin grafts. Those having ordinary skill in the art can readily identify individuals suspected of suffering from or being susceptible to organ and/or tissue

rejection. Those with ordinary skill in the art could readily identify individuals for whom administration of the compounds of the invention would be beneficial to alleviate or prevent immune response associated with organ/tissue rejection. Treatment may be provided prophylactically in conjunction with transplantation procedure or in response to symptoms associated with organ or tissue rejection. Pharmaceutical compositions useful in the methods of the present invention comprise the compounds described herein.

The method of therapeutically or prophylactically treating an individual suffering from or susceptible to organ or tissue rejection comprises administering to such an individual an effective amount of a peptide according to the invention. A prophylactically effective amount is an amount which is effective to prevent or decrease the immune response associated with organ and tissue rejection in an individual susceptible to organ or tissue rejection. A therapeutically effective amount is an amount which is effective to decrease or eliminate organ or tissue rejection in an individual suffering from organ or tissue rejection. Those having ordinary skill in the art can readily and routinely determine the ranges of both prophylactically and therapeutically effective amounts of the peptides of the invention without undue experimentation.

The present invention provides pharmaceutical compositions that comprise the peptides of the invention and pharmaceutically acceptable carriers or diluents. The pharmaceutical composition of the present invention may be formulated by one having ordinary skill in the art. Suitable pharmaceutical carriers are described in *Remington's Pharmaceutical Sciences*, A. Oslo, a standard reference text in this field, which is incorporated herein by reference. In carrying out methods of the present invention, conjugated compounds of the present invention can be used alone or in combination with other diagnostic, therapeutic or additional agents. Such additional agents include excipients such as coloring, stabilizing agents, osmotic agents and antibacterial agents.

For parenteral administration, the peptides of the invention can be, for example, formulated as a solution, suspension, emulsion or lyophilized powder in association with a pharmaceutically acceptable parenteral vehicle. Examples of such vehicles are water, saline, Ringer's solution, dextrose solution, and 5% human serum albumin. Liposomes and nonaqueous vehicles such as fixed oils may also be used. The vehicle or lyophilized powder may contain additives that maintain isotonicity (e.g., sodium

chloride, mannitol) and chemical stability (e.g., buffers and preservatives). The formulation is sterilized by commonly used techniques. For example, a parenteral composition suitable for administration by injection is prepared by dissolving 1.5% by weight of active ingredient in 0.9% sodium chloride solution.

The pharmaceutical compositions according to the present invention may be administered as a single dose or in multiple doses. The pharmaceutical compositions of the present invention may be administered either as individual therapeutic agents or in combination with other therapeutic agents. The treatments of the present invention may be combined with conventional therapies, which may be administered sequentially or simultaneously. In some embodiments, compounds are administered 1-2 days prior to transplantation, preferably 4-12 hours. Compounds may be delivered during transplantation procedures. In some embodiments, compounds are administered for 2 weeks to 2 months after transplantation procedures.

The pharmaceutical compositions of the present invention may be administered by any means that enables the active agent to reach the targeted cells. These methods include, but are not limited to, oral, topical, intradermal, subcutaneous, intravenous, intramuscular and intraparenteral modes of administration. The compounds may be administered singly or in combination with other compounds. The compounds of the invention are preferably administered with a pharmaceutically acceptable carrier selected on the basis of the selected route of administration and standard pharmaceutical practice.

The dosage administered varies depending upon factors such as: pharmacodynamic characteristics; its mode and route of administration; age, health, and weight of the recipient; nature and extent of symptoms; kind of concurrent treatment; and frequency of treatment. Usually, the dosage of peptide can be about 1 to 3000 milligrams per 50 kilograms of body weight; preferably 10 to 1000 milligrams per 50 kilograms of body weight; more preferably 25 to 800 milligrams per 50 kilograms of body weight. Ordinarily 8 to 800 milligrams are administered to an individual per day in divided doses 1 to 6 times a day or in sustained release form is effective to obtain desired results.

EXAMPLES

The following examples are merely illustrative of the present invention and should not be considered limiting of the scope of the invention in any way. These examples and equivalents thereof will become more apparent to those skilled in the art in light of the present disclosure and the accompanying claims.

Example 1 Inhibition of cytotoxic T-cell mediated lympholysis by peptide in a human CML

The effect of the peptides on cytotoxicity was measured in a 4-hour ^{51}Cr - release assay. Specific effectors were generated in mixed lymphocyte reaction from two different human donors. The stimulators [human PBL - donor 1] were gamma-irradiated at 3000 RADS. The targets were obtained from PHA stimulated donor 1 PBLs that were labeled with $Na^{51}CrO4$. On Day 6 the effectors are harvested and counted. The peptide to be tested (at concentrations of 100, 10 or 1 microgram per milliliter (μg/ml)) was added to effectors of varying numbers and then ^{51}Cr-labeled targets were added in a 4-hour specific lysis experiment. The target cell lysis was assessed at Effector:Target ratios of 50: 1, 25:1, and 10:1. Toxic effects of the peptide were determined by the % specific lysis above background that was obtained by incubating just peptide and labeled targets. No toxic effects were observed. Also, anti-CD8 monoclonal antibody that blocks the interaction resulting in lowered lympholysis was used in the assay.

The peptides generated from the D1 domain of the CD8α-chain (human) and their efficacy in inhibition of CD8 activation are listed in Table 1.

Table 1: Peptide Efficacy in inhibiting CD8 activation

PEPTIDE NO.	hCD8 LOOP	PEPTIDE SEQUENCE	INHIBITION CML (%)
1109	DE	CKRLGDTFVC SEQ ID NO:31	82.1
1169	DE LINEAR	KRLGDTFV SEQ ID NO:45	4.2
1168	DE-SCR	CVGTFRKDLC	40.6

1127	mut DE	CKRAGDTFVC SEQ ID NO:46	57.4
1128	mut DE	CKRLGATFVC SEQ ID NO:47	52.2
1129	truncated DE	CLGDTC SEQ ID NO:30	17.3
1130	mut DE	CKRLGDAFVC SEQ ID NO:62	11.7

These peptides were tested for their ability to inhibit CD8-dependent CTL lysis of target cells in a standard 4-hour chromium release assay using human PBLs.

The biological activity of 1109 was extensively tested by replacement of critical amino acids with alanine and then testing for the ability to inhibit lysis in a CML. These alanine scan peptides (1127, 1128 and 1330) all showed diminished activity indicating that these residues were important for the biological activity of the parent peptide 1109.

The data indicate that 1109 was the best at inhibiting CD8 dependent CTL lysis as tested in Human CML assays. To further test the specificity of the peptide 1109, a scrambled and a linear form of the peptide was tested in vitro in the human CML assay. Both showed less ability to inhibit CTL lysis than 1109, as seen in Table 1.

Example 2 In vivo testing

Peptide 1109 was also tested in vivo in a CD8-dependent MHC class 1-restricted mouse skin allograft model. Preliminary data suggests that a single dose of 0.5 mg at the time of transplantation is sufficient to significantly prolong allograft survival.

Tail skin from C57bl/7 [H-2B] donor was grafted onto the ventral side of bm1 recipient tails. The donors were asphyxiated and 2 x 4 mm tail pieces are cut. These grafts were placed in 0.1% BSA in PBS on ice. Recipients were anesthetized in isoflurane chamber. While still anesthetized, grafting beds of 10 x 10 mm on the ventral side of the tail were cut. Two grafting beds per mouse were made, one for syngeneic graft and the other for the allogeneic graft. The grafts were turned 180° and placed in the beds such that

the hair of the graft would grow in the opposite direction. The grafts were trimmed if necessary such that they fit in the beds without skin hanging over the edge of the beds. A blunt glass tube was placed over the grafts and taped into place. This glass tube was removed on day 2 post-transplantation.

Five animals per treatment group were used and treatment was prior to transplantation. The untreated group was injected in the tail vein with 200 microliters (µl) of PBS on Day 0. The anti-CD8 antibody treatment group was injected i.p. with 200 µl of 1:5 dilution of 2.43 ascites on Day 0. The 1109 peptide group was injected in the tail vein with 0.5 mgs/200 µl of PBS on Day 0.

Grafts were observed every other day for signs of rejection. Rejection was indicated when the graft showed loss of hair, loss of pigmentation, and development of scar tissue. Grafts were considered rejected due to mechanical failure if the grafts were rejected by Day 3. The acceptance of syngeneic grafts was 90% or better for each experiment. 50% of the allogeneic grafts were rejected by Day 12-15 in untreated groups. Rejection was significantly delayed in peptide treated groups.

Example 3

Peptides were designed using sequence information derived from the sequence of the α chain of the CD8 protein. These peptides were synthesized and observed to inhibit the CD8-dependent activation pathways of the CTL. Two "active" loop regions have been identified. In the human CD8 the two loops are centered in and around residues Asn 55-Lys 56 and residues Leu 63-Asp 64. The corresponding murine equivalent loops are located in and around murine residues Ser 55-His 56 and Leu 66-Asn 67, respectively.

The following peptides were synthesized based on the corresponding murine sequences:

1) C-S-S-H-N-K-P-C, SEQ ID NO:7 (referred to as SC2; including murine sequences 54-58); and

2) C-D-E-K-L-N-S-S-K-L-C, SEQ ID NO:8 (referred to as SC11; murine sequences 63-71). The underlined portion of these sequences refer to the sequences derived from the murine CD8 protein.

The murine peptides parallel the active regions of human peptides. Experiments are performed in murine models to test the pharmacological activity of the murine peptides. These results are comparable to the use of parallel peptides based upon human sequences for the treatment of human conditions.

Example 4 Inhibition of cytotoxic T-cell mediated lympholysis by peptide

The effect of peptide on cytotoxicity was measured in a 4 hour ^{51}Cr release assay. H-2^b specific effectors were generated in a AKR/J (H-2^k) anti C57BL/6 (H-2^b) mixed lymphocyte reaction. The stimulators (C57BL/6 spleen cells) are gamma-irradiated at 1500 RADS. The targets were obtained from a tumor cell line, EL-4 (H-2^b), that were labeled with $Na^{51}CrO_4$. On Day 5 the effectors are harvested and counted. The peptide, at concentrations of 100 µg/ml, 10 µg/ml or 1 µg/ml, was first incubated for 30 minutes at 37°C with effectors of varying numbers and then ^{51}Cr labeled targets were added in a 4 hour specific lysis experiment. The target cell lysis was assessed at Effector:Target ratios of 30:1, 10:1, 3:1, and 1:1. Toxic effects of the peptide was determined by the % specific lysis above background that was obtained by incubating just peptide and labeled targets. No toxic effects were observed. Also, anti-CD8 monoclonal antibody that blocks the interaction resulting in lowered lympholysis is used in the assay. SC2 inhibits specific lysis of targets in a concentration dependent manner and SC11 does not.

Example 5 Inhibition of activation/generation of cytotoxic T-cells by peptide

The effect of the peptide on the activation of CTL capable of lympholysis was determined by generating effector T-cells in AKR/J anti C57BL/6 mixed lymphocyte reaction in the presence of peptide. On Day-6, effectors are washed and then ^{51}Cr-labeled EL-4 targets were added in a 4-hour specific lysis experiment. Both SC2 and SC11 show inhibition of specific lysis of targets in a concentration dependent manner indicating that they may be preventing the induction of activated CTL.

Example 6. SC2 murine CD8 DOMAIN 1 peptide 54-59: CSSHNKPC (SEQ ID NO:7) MWt 869.97

SC2 has been used in murine skin grafting experiments and also in murine GVHD model. The data generated in these experiments showed that SC2 consistently prolonged skin graft survival with biological significance. Data also showed that SC2 delays the onset of GVHD in a murine GVHD model that is primarily CD8+ based.

Experimental Method:

Skin Transplantation

Tail skin from C57BL/7 [H-2b] donor is grafted onto the ventral side of bm1 recipient tails. The donors are asphyxiated and 2 X 4 mm tail pieces are cut. These grafts are placed in 0.1% BSA in PBS on ice. Recipients are anesthetized in isoflurane chamber. While still anesthetized, grafting beds of 10 X 10 mm on the ventral side of the tail are cut. Two grafting beds per mouse are made, one for syngeneic graft and the other for the allogeneic graft. The grafts are turned 180' and placed in the beds such that the hair of the graft grows in the opposite direction. The grafts are trimmed if necessary such that they fit in the beds without skin hanging over the edge of the beds. A blunt glass tube is placed over the grafts and taped into place. This glass is removed on day 2 post-transplantation.

Five animals per treatment group are used and treatment is prior to transplantation. The untreated group is injected into the tail vein with 200 µl of PBS on Day 0. The anti-CD8 antibody treatment group is injected i.p. with 200 µl of 1:5 dilution of 2.43 ascites on Day 0. The SC2 peptide group is injected in the tail vein with 0.5 mgs/200 µl of PBS on Day 0.

Grafts are observed every other day for signs of rejection. Rejection is when the graft shows loss of hair, loss of pigmentation, and development of scar tissue. Grafts are considered rejected due to mechanical failure if the grafts are rejected by Day 3. The acceptance of syngeneic grafts is 90% or better for each experiment. The 50% of the allogeneic grafts are rejected by Day 12-15 in untreated groups. Rejection is significantly delayed in peptide treated groups.

Murine Model of GVHD

Donor bone marrow is harvested from tibias and femurs of B10.BR mice. T-cells are eliminated from the bone marrow by treatment with anti-Thy1.2 and complement. Complement treatment for 2 cycles at 37°C for 1 hour each results in more than 90% pure population of bone marrow cells. 10 X 10^6 T-cell depleted bone marrow (ATBM) cells are

injected into the tail vein. Donor splenocytes undergo red blood cell lysis followed by B cell and CD4+ T-cells are depletion using anti-J1J and anti-RL147 antibodies, respectively. This purified CD8+ T-cell population from donors (5 X 10^6 cells) is injected into the tail vein of the animal. The host is lethally irradiated at 850 cGy approximately 6 hours prior to bone marrow transplantation. Characteristic features of murine acute GVHD include weight loss, diarrhea and loss of hair and death. Body weights were monitored before and after treatment. The treated mice were housed in sterile conditions and observed daily for acute GVHD-like reactions. Lethally irradiated mice reconstituted with ATBM only survive without signs of disease. Mice are treated with 0.5 mgs of SC2 peptide on Day 0, 3 and 6.

Example 7 Inhibition of cytotoxic T-cell mediated lympholysis by peptide Wu-1 HUMAN CD8 PEPTIDE: CLSQNKPC (SEQ ID NO:11) MWt 887.04

Wu-1 (SEQ ID NO:10) is a cyclic peptide consisting of human CD8 amino acids 52-57 (SEQ ID NO:9) with amino terminal and carboxy terminal cysteines to yield an eight amino acid peptide (SEQ ID NO:10). Wu-1 was tested in the ^{51}[Cr]-release assay as described in Example 2. Data showed that Wu-1 showed approximately 50% inhibition of killing.

Example 8

Wu-2 is a linear peptide consisting of human CD8 amino acids 37-47 (SEQ ID NO:12). Wu-2 was used in the skin transplantation assay described in Example 4 and was observed to cause a significant delay in rejection in skin transplantation experiments.

Various modifications of the invention, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

The disclosures of each patent, patent application, and publication cited or described in this document are hereby incorporated herein by reference in their entirety.

Example 9

The effect of the CD8 inhibitor 1109 was tested in the CD8-dependent MHC class I - restricted skin transplantation model. The data, which is shown in Figure 1, demonstrates that Peptide 1109 effectively prolongs the survival of allografted bm1 tail skin with MST of 28 days as compared to the PBS control (MST=17 days). Peptide 1255 is the scrambled control of 1109 and had an effect similar to that of PBS. The data also shows that anti-CD8 mAb 2.43 also significantly prolongs graft survival with 40% engrafted at day 60.

The peptide 1109 was also tested for its ability to inhibit GVHD in minor histocompatibility mismatch which is primarily CD8+ T cell mediated. Mice were i.v. injected with 15 x 10^6 CD8+ T cells and 2 x 10^6 T cell depleted bone marrow (ATBM) and peptide treatment. The data is shown in Figure 2. Animals that received 0.5 mg of 1109 on day 0 showed prolonged survival when compared to untreated and scramble peptide, 1255-treated groups.

What is claimed is:

1. A conformationally restricted peptide consisting of 4 to 25 amino acids including one of SEQ ID NOs:15-28, wherein said peptide inhibits CD8 mediated T cell activity.

2. The conformationally restricted peptide of claim 1 having the formula

R_{101}-R_{102}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

3. The conformationally restricted peptide of claim 2 wherein said linking moieties R_{101} and R_{110} are each independently cysteine, phenylalanine, penicillamine, or proline.

4. The conformationally restricted peptide of claim 2 wherein at least one of said linking moieties is cysteine.

5. The conformationally restricted peptide of claim 1 having the formula:

R_{101}-R_{104}-R_{105}-R_{106}-R_{107}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine; and

R_{110} is a linking moiety.

6. The conformationally restricted peptide of claim 5 wherein said linking moieties R_{101} and R_{110} are cysteine, phenylalanine, penicillamine, or proline.

7. The conformationally restricted peptide of claim 1 having the formula:

R_{101}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine; and

R_{110} is a linking moiety.

8. The conformationally restricted peptide of claim 1 wherein said linking moieties R_{101} and R_{110} are cysteine, phenylalanine, penicillamine, or proline.

9. A conformationally restricted peptide of claim 1 comprising one of SEQ ID NOs:15-28.

10. A conformationally restricted peptide of claim 1 selected from the group consisting of: SEQ ID NO:29, SEQ ID NOs:31-44, SEQ ID NOs:46-60 and SEQ ID NO:63.

11. A pharmaceutical composition comprising:

a) a conformationally restricted peptide of claim 1 and

b) a pharmaceutically acceptable carrier or diluent.

12. The pharmaceutical composition of claim 11 wherein said conformationally restricted peptide has the formula

R_{101}-R_{102}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

13. The pharmaceutical composition of claim 11 wherein said conformationally restricted peptide comprises SEQ ID NO:29, SEQ ID NOs:31-44, SEQ ID NOs:46-60 and SEQ ID NO:63.

14. A method for inhibiting activation of a human T cell comprising contacting said cell with a peptide of claim 1.

15. The method of claim 14 wherein said peptide has the formula

R_{101}-R_{102}-R_{103}-R_{104}-R_{105}-R_{106}-R_{107}-R_{108}-R_{109}-R_{110}

wherein:

R_{101} is a linking moiety;

R_{102} is lysine, arginine, valine or histidine;

R_{103} is arginine, lysine, glycine or glutamine;

R_{104} is alanine, leucine, isoleucine, threonine or valine;

R_{105} is glycine;

R_{106} is aspartic acid, alanine, glutamic acid or asparagine;

R_{107} is threonine, alanine or serine;

R_{108} is phenylalanine, tyrosine, tryptophan or valine;

R_{109} is valine, leucine or isoleucine; and

R_{110} is a linking moiety.

16. The method of claim 14 wherein said peptide comprises SEQ ID NO:29, SEQ ID NOs:31-44, SEQ ID NOs:46-60 and SEQ ID NO:63.

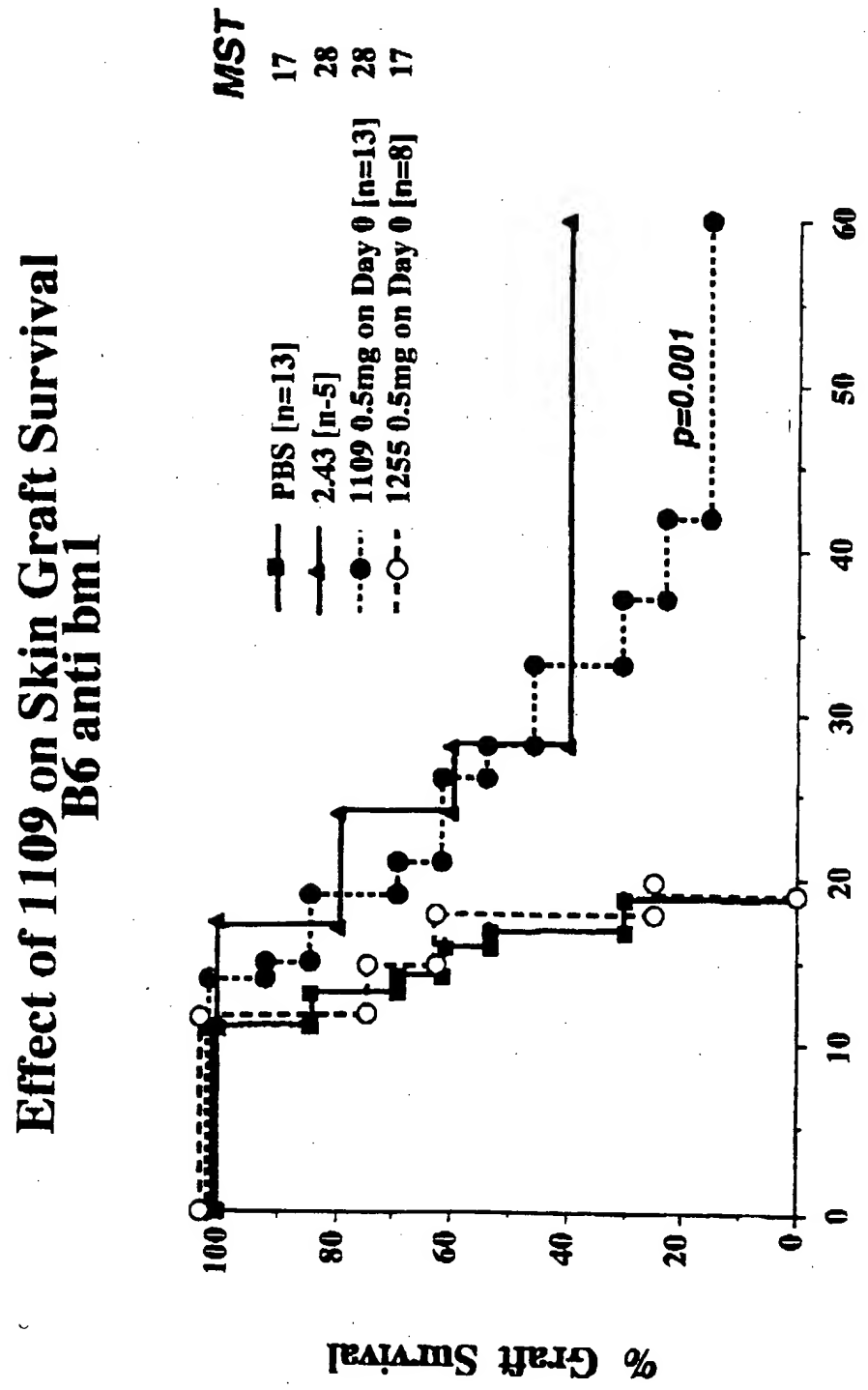
Effect of 1109 on Skin Graft Survival
B6 anti bm1
MST
PBS [n=13] 17
2.43 [n-5] 28
1109 0.5mg on Day 0 [n=13] 28
1255 0.5mg on Day 0 [n=8] 17
p=0.001
% Graft Survival
Day
0
20
40
60
80
100
0
10
20
30
40
50
60

FIGURE 1

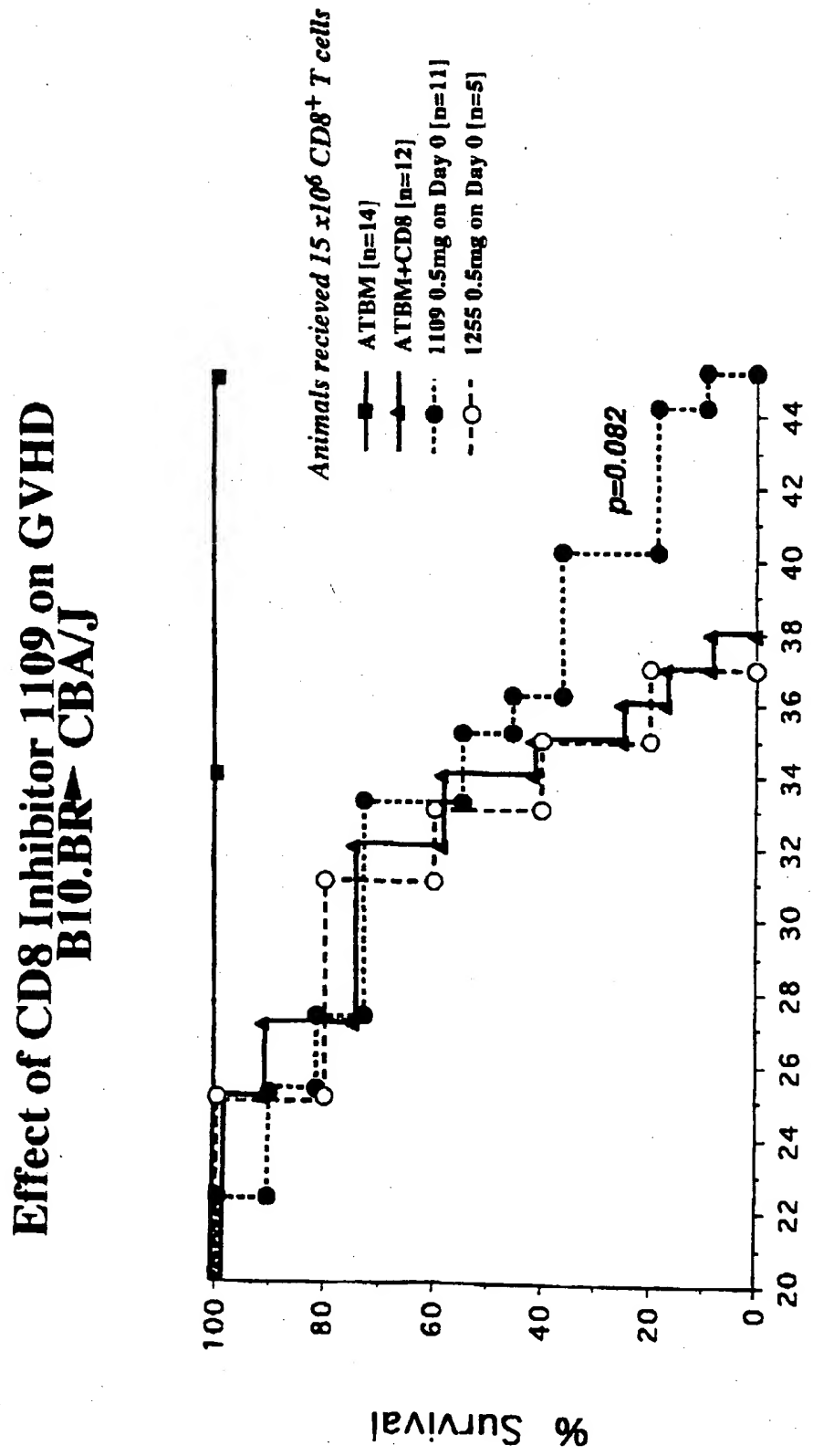
Effect of CD8 Inhibitor 1109 on GVHD
B10.BR→ CBA/J
Animals recieved 15 x10⁶ CD8⁺ T cells
ATBM [n=14]
ATBM+CD8 [n=12]
1109 0.5mg on Day 0 [n=11]
1255 0.5mg on Day 0 [n=5]
p=0.082
% Survival
Day
0
20
40
60
80
100
20
22
24
26
28
30
32
34
36
38
40
42
44

FIGURE 2

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20 25 30

Cys Ser Trp Leu Phe Gln Pro Arg Gly Ala Ala Ala Ser Pro Thr Phe
35 40 45

Leu Leu Tyr Leu Ser Gln Asn Lys Pro Lys Ala Ala Glu Gly Leu Asp
50 55 60

Thr Gln Arg Phe Ser Gly Lys Arg Leu Gly Asp Thr Phe Val Leu Thr
65 70 75 80

Leu Ser Asp Phe Arg Arg Glu Asn Glu Gly Tyr Tyr Phe Cys Ser Ala
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Leu Ser Asn Ser Ile Met Tyr Phe Ser His Phe Val Pro Val Phe Leu
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<220>
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<400> 63
Cys Lys Leu Gly Asp Thr Phe Val Cys
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08814

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 5/00,
US CL :530/300

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/300

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

protein sequence database

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PETANCESKA et al. Sequence Analysis, Tissue Distribution, and Expression of Rat Cathepsin S. J. American Society for Biochemistry and Molecular Biology. July 1992, Vol. 267, pages 26038-26043, see entire document.	1

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 16 JUNE 1999	Date of mailing of the international search report 20 AUG 1999
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Tara Custer JOYCE BRIDGERS PARALEGAL SPECIALIST CHEMICAL MATRIX Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08814

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/08814

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains claims directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for more than one species to be searched, the appropriate additional search fees must be paid. The species are as follows:
Group I: Claim 1, drawn to species: 1-14: SEQ. ID NO. 15-28 (each sequence is a different species)

Group II: Claims 2-13, drawn to a conformationally restricted peptide and a pharmaceutical composition

Group III: Claims 14-16, drawn to a method for inhibiting activation of a human T cell.

The following claims are generic: 1
The species listed above do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, the species lack the same or corresponding special technical features for the following reasons:
The technical feature linking groups I-III appears to be that they all related to a peptide comprising a SEQ. selected from 15-28. However, SEQ ID. NO. 15 is taught by Petanceska et al. (J. of Biol. Chem. 1992; Vol. 267; pages 26038-26043. Therefore, the technical feature linking the inventions of groups I-III does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of group I is considered to be a peptide consisting of 4 to 25 amino acids of SEQ. ID. NOs. 15-28, wherein the peptide inhibits T cell activity.

The special technical feature of group II is considered to be a peptide consisting of 4 to 25 amino acids of SEQ. ID. NOs. 15-28, and a pharmaceutical composition.

The special technical feature of group III is considered to be a peptide consisting of 4 to 25 amino acids of SEQ. ID. NOs. 15-28, wherein the peptide inhibits T cell activity by contacting T cells.

Accordingly, Groups I-III are not so linked by the same or a corresponding special technical feature as to form a single general inventive concept.